Helix–Coil Transition in Mixed Solvents. I. Optical Rotatory Dispersion Study of Poly(γ-benzyl L-glutamate) in Dichloroacetic Acid–Dichloroethane Mixtures[1]

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ABSTRACT: The Zimm–Bragg parameters σ and s for the helix–coil transition of poly(γ-benzyl L-glutamate) in mixtures of dichloroacetic acid and dichloroethane containing 70 and 82 wt % of dichloroacetic acid, respectively, have been determined by spectropolarimetric measurements of the helix content of the polypeptide as a function of temperature, using fractionated samples of varying average chainlengths. The experimental data are fit well using a temperature-independent value of σ. The changes in free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy ($\Delta S°$) for the conversion of the poly(amino acid) residue from the coil to the helix form have been calculated in each solvent composition from the temperature dependence of s. The values obtained for $\Delta H°$ in the two solutions are found to be smaller than published data but seem to compare fairly well with the calorimetric values presented in the accompanying paper. Furthermore, the transition curves, and therefore presumably $\Delta H°$, are independent of the concentration of the poly(amino acid) over the concentration range studied.

In a parallel series of investigations,[3-9] we are determining the conformational preferences of the naturally occurring amino acids in water by evaluating the Zimm–Bragg parameters[10] σ and s for the guest residues in random copolymers formed from these residues and suitable host residues. In order to obtain the conformational preferences of the amino acids in nonaqueous solvents, a similar approach can be taken, in which poly(γ-benzyl L-glutamate) (PBLG) can serve as the host. This paper, and the accompanying one,[11] are the initial ones, in which the host homopolymer in a mixture of dichloroacetic acid ($HCCl_2COOH$) and dichloroethane (Cl_2Et) is characterized.

A mixed solvent is used here (one of which favors the helical conformation and the other the coil one) because most poly(amino acids) do not undergo a *thermally* induced helix–coil transition in pure organic solvents.[12] For ease in analysis of the experimental data, σ and s are best determined from a thermally induced transition in a system of fixed solvent composition.

Since the water-soluble random copolymers used in our related investigations[3-9] are derived from PBLG, this polymer was selected as the host for the studies in nonaqueous solvents. While the helix–coil transition in $HCCl_2COOH$–Cl_2Et mixtures (in which the transition is an inverted one, *i.e.*, increase of temperature induces formation of helix[13]) has been the subject of many earlier investigations,[13-24] there is not general agreement among the data reported from various laboratories. Because the host homopolymer must be characterized in a consistent manner with any random copolymers derived from it, we report here, and in the accompanying paper,[11] the properties of this system as determined by optical rotatory dispersion (ORD) and calorimetric measurements.

Experimental Section

A. Materials. Several samples of PBLG with varying chainlengths ($\overline{DP}$'s) were synthesized by the *N*-carboxyanhydride method[25] using dioxane as the solvent and triethylamine as the initiator. The solvent and the initiator were purified as described earlier.[4] Dimethylformamide used for the molecular weight determinations was a Fisher Spectranalyzed sample, while Cl_2Et was of Spectrograde quality from Mallinckrodt. The ultraviolet absorption spectrum of Cl_2Et was checked periodically to assess its purity. $HCCl_2COOH$ was obtained as a Fisher purified sample, and was distilled usually once but sometimes twice under reduced pressure (~20 mm) and stored at low temperature (4°), but no longer than 2 weeks. The purity of the distilled $HCCl_2COOH$ was checked both by refractive index and ultraviolet absorption measurements; the former was not very sensitive to small amounts of impurities detected by the latter method.

B. Methods. The fractionation of the PBLG samples was done by fractional precipitation of the polymer from a solution in dioxane, using ethanol as the precipitant. With the highest molecular weight PBLG samples three fractions were obtained, the large middle fraction being chosen for the ORD experiments. With samples of lower $\overline{DP}$'s, a larger number of fractions was obtained (usually five) and the central fractions were selected for ORD studies. The fractions were isolated by centrifugation at 2000 rpm, dissolved in dioxane, lyophilized, and dried over P_2O_5 under high vacuum.

The weight-average molecular weights ($\overline{M}_w$) of the fractions selected for the ORD measurements were determined in the Beckman Model E ultracentrifuge with interference optics, using dimethylformamide as the solvent. In this solvent, PBLG is as disaggregated as it is in $HCCl_2COOH$.[26-28] Kel-F centerpieces were employed for the synthetic boundary and equilibrium measure-

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Table I
Molecular Weights of PBLG Fractions Used for ORD Experiments

Fraction[a]	$\bar{M}_w{}^{\circ\, b} \times 10^{-4}$	$\overline{DP}_w$
I-2[c]	34.0	1550 ± 155
II-2[c,d]	21.9	1000 ± 100
III-3[d]	15.3	700 ± 50
III-6[c,d]	7.0	320 ± 16
IV-3[c,d]	2.9	130 ± 7

[a] The Roman numerals refer to the individual samples of PBLG synthesized, while the arabic numbers denote the fraction number. [b] Weight-average molecular weight extrapolated to zero concentration of PBLG. [c] Used for ORD measurements in 82 wt % $HCCl_2COOH$ solution. [d] Used for ORD measurements in 70 wt % $HCCl_2COOH$ solution.

ments. The partial specific volume, $\bar{v}$, of PBLG in dimethylformamide was taken[29] as 0.786 ml/g and used in the calculation of $\bar{M}_w$ by the sedimentation equilibrium method. As expected on the basis of the earlier studies,[28] a dependence of $\bar{M}_w$ on PBLG concentration in the dimethylformamide solution, arising from nonideality of the solution, was observed, the apparent $\bar{M}_w$ increasing with decreasing polymer concentration in the range of 0.1–0.5% (w/v). The weight-average molecular weight at zero concentration of polymer ($\bar{M}_w{}^0$) was obtained for all fractions by extrapolation of the values of $\bar{M}_w$ at the several concentrations where they were determined by the conventional sedimentation equilibrium method.[30] To facilitate such an extrapolation for the fractions of higher $\overline{DP}$ ($\bar{M}_w > 2 \times 10^5$), which showed a pronounced nonideal effect, the values of $\bar{M}_w$ were obtained at very low concentrations, *viz.*, 0.01–0.05% (w/v), using the meniscus depletion method of Yphantis.[31] The data were analyzed by the usual procedure.[30] In the case of all the fractions studied, the analysis of the data to obtain the ratio, $\bar{M}_z{}^0/\bar{M}_w{}^0$, where $\bar{M}_z{}^0$ is the *z*-average molecular weight at infinite dilution, yielded values for this ratio close to unity (less than 1.05), indicating the absence of any significant polydispersity in the fractionated samples. (It is possible, however, that the effects of nonideality, when present, and polydispersity may fortuitously cancel each other and give low values of $\bar{M}_z{}^0/\bar{M}_w{}^0$; however, the nonideal effect would be expected to diminish rapidly at lower polymer concentration while the effect of polydispersity would not.) Table I gives the values of $\bar{M}_w{}^0$ of the five PBLG fractions used in the ORD experiments. In the case of fractions I-2 and II-2, the molecular weights calculated from viscosity measurements in $HCCl_2COOH$, using the intrinsic viscosity–molecular weight relationship of Doty *et al.*,[26] were found to be 1370 and 910, respectively, which are close to the values of $\bar{M}_w{}^0$ listed in Table I. The error in $\bar{M}_w{}^0$ ranged from ±10% for I-2 and II-2, to ±7% for III-3 and ±5% for III-6 and IV-3. It may be noted that the relatively larger error for the two samples of higher $\overline{DP}$ is not very significant inasmuch as the transition curves are expected and, as shown later, are found to be much less sensitive to variation in $\overline{DP}$ at high values of $\overline{DP}$ than at lower values of $\overline{DP}$.

The ORD measurements were made on a Cary Model 60 spectropolarimeter. Water-jacketed quartz cells of 1.0- and 2.5-cm path lengths were used in conjunction with a Haake thermostated circulating water bath with a temperature control of ±0.2°. The temperature was measured with the aid of a calibrated thermistor probe situated close to the cell. Because of thermal convection in the dense solvent mixtures used, a sufficiently long time was allowed for thermal equilibrium at each temperature in order to obtain reproducible tracings of the ORD curves. Dry nitrogen was circulated to eliminate moisture from the cell compartment at low temperatures. The ORD data were fit to the Moffitt-Yang equation[32] to obtain values of the parameter b_0 for PBLG in the solvent mixture. Data in the wavelength region 300–480 mμ were used for this purpose, and a value of 212 mμ was used for the constant λ_0 of the Moffitt-Yang equation. The measured mean residue rotation at each wavelength was corrected for the refractive index of the solvent mixture at that wavelength (calculated from data on the dispersion of the refractive index of $HCCl_2COOH$ and Cl_2Et[33] using the Sellmeir equation[34]). In addition, correction was also made for the change in concentration of the polymer due to the change in volume of the solvent mixture at different temperatures, using data for the temperature dependence of the densities of $HCCl_2COOH$ and Cl_2Et.[35] The average error in the determination of b_0 from the slope of the least-squares Moffitt-Yang plot was estimated to be ±2.5 units in b_0 throughout the range of values of b_0.

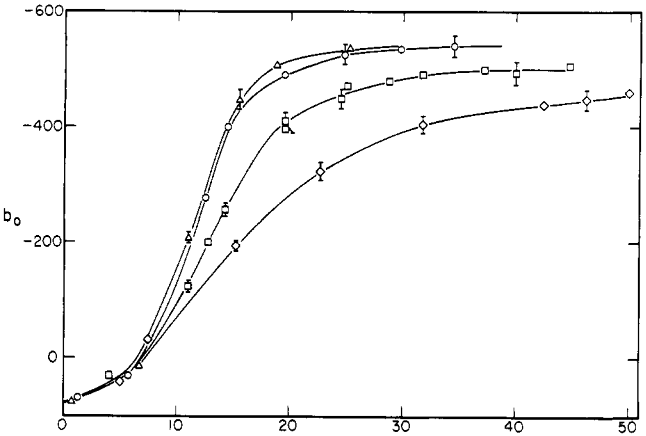

Figure 1. Plot of b_0 *vs.* *T* for PBLG in 70 wt % $HCCl_2COOH$ solution: (△) fraction II-2, $\overline{DP}$ = 1000; (○) fraction III-3, $\overline{DP}$ = 700; (□) fraction III-6, $\overline{DP}$ = 320; (◇) fraction IV-3, $\overline{DP}$ = 130. Error limits are indicated by vertical lines.

The concentrations of the PBLG solutions in the solvent mixtures generally ranged from about 0.35 to 2.5% (w/v); in addition, a relatively high concentration of 4% was used in the case of fraction II-2 to check the concentration dependence of the transition curve. The concentration of PBLG in the solution was determined in most cases from the known weight of the dry polymer dissolved in a known volume of the solvent mixture (assuming that the volume of the solution is equal to that of the solvent mixture). In several cases, however, the concentration was, in addition, estimated by micro-Kjeldahl nitrogen analysis,[36] after first evaporating the solvents in a vacuum oven at 100° or above. Within the limits of experimental error of the micro-Kjeldahl method (±3%), the concentrations obtained were identical with those calculated from the direct-weighing procedure. The solvent mixture was made up in terms of the weight percentage (wt %) of $HCCl_2COOH$ in the mixture; *i.e.*, a 70 wt % $HCCl_2COOH$ solution contains 70 g of $HCCl_2COOH$ and 30 g of Cl_2Et in 100 g of the mixture. The 70 and 82 wt % $HCCl_2COOH$ solutions used here correspond to 65.2 and 78.5% v/v, respectively.

Results

The thermally induced conformational transition of PBLG from the coil form at low temperatures to the helical form at high temperatures (*i.e.*, the "inverted" helix-coil transition[10]) was studied at two compositions of the $HCCl_2COOH$-Cl_2Et solvent mixture, *viz.*, 70 and 82 wt % $HCCl_2COOH$ solutions, using the fractionated PBLG samples listed in Table I. The course of the transition was followed by the change in the value of b_0 of the polypeptide solution with temperature (see Figures 1 and 2). The transition was found to be completely reversible, as was first observed by Doty and Yang.[13] For a given composition of the solvent mixture, the b_0 *vs.* temperature curve was independent of the PBLG concentration over the

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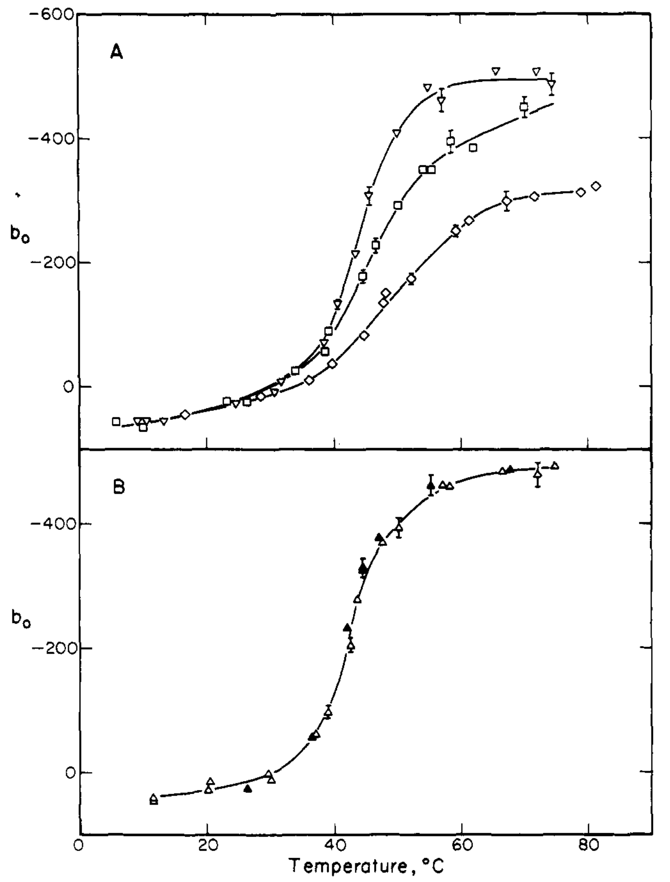

Figure 2. Plot of b_0 *vs.* T for PBLG in 82 wt % $HCCl_2COOH$ solution. (A) (▽) Fraction I-2, $\overline{DP}$ = 1550; (□) fraction III-6, $\overline{DP}$ = 320; (◇) fraction IV-3, $\overline{DP}$ = 130. (B) Fraction II-2, $\overline{DP}$ = 1000; the filled symbols in B correspond to a PBLG concentration of 4% (w/v) while the open symbols correspond to PBLG concentration of 0.4 and 2.5% (w/v). Error limits are indicated by vertical lines.

range of 0.35-2.5%. At a higher concentration of 4.0% used in one case (*viz.* fraction II-2), the transition curve was shifted by only about 1° to lower temperatures (see Figure 2B). The error symbols shown for the b_0 values in the figures (omitting errors in temperature and solvent composition) include errors in the determination of the concentration of the PBLG solutions (±3%), and in the evaluation of the slope of the Moffitt-Yang plot (2.5 units in b_0).

The values of b_0^H and b_0^C, for the complete helix and complete coil, respectively, required for the computation of the fractional helix content θ_h from b_0, were obtained as follows. A value of $b_0^H = -700$ was chosen from measurements (not shown here) on the high-$\overline{DP}$ fractions I-2 and II-2 in pure Cl_2Et solution at 25°;[37] the same value of b_0,[4] also based on a high-$\overline{DP}$ sample of PBLG in pure Cl_2Et, was used by Norisuye *et al.*[22] From measurements in $HCCl_2COOH$, we obtained a value of $b_0^C = +75$, which is similar to that shown in Figures 1 and 2 for $HCCl_2COOH$-Cl_2Et mixtures at low temperature; this value is slightly larger than that found by Norisuye *et al.*[22] The actual value used here for b_0^C was selected in two ways. In one of them, the value of 75 was taken to represent b_0^C throughout the transition, and θ_h was then calculated as $-(b_0 - 75)/775$. In the second method, it was assumed that the b_0^C value of 75 corresponds to the coil form of the polymer to which $HCCl_2COOH$ molecules are bound, and thus may not correspond to the unsolvated coil form of PBLG, which then was assumed to have a value of $b_0^C = 0$ as in other cases.[4-9] (It may be noted in this connection that the ORD curve for PBLG in hydrazine, which promotes the coil form, differed from the curve for PBLG in $HCCl_2COOH$,[13] with $b_0^C = 0$ in hydrazine.) The value of b_0^C was therefore made to vary linearly from 75 to 0 throughout the transition as θ_h changed from 0 to 1. θ_h was thus calculated as $[b_0 - (1 - \theta_h) \times 75]/[-700 - (1 - \theta_h) \times 75]$. However, the values calculated for θ_h by the above two procedures did not differ very much from each other, and the same final values of the thermodynamic parameters (within the limits of error) were obtained by both procedures. Therefore, the first procedure was used routinely. The error involved in computing θ_h should include not only the errors in b_0 mentioned earlier but also the possible error in the limiting values of b_0^H and b_0^C. Since the errors in b_0^H and b_0^C were not determinable directly, an allowance of ±3% for b_0^H and ±9% for b_0^C (based on the reproducibility of these values) produced an error of ±2.5% in θ_h at a b_0 value of -300, corresponding to about the middle of the transition; this value was incorporated in the computed error in θ_h for all values of θ_h.

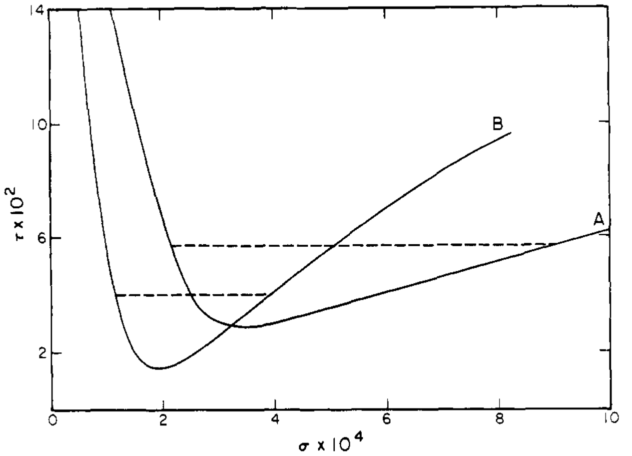

Figure 3. Plot of τ *vs.* σ. (A) in 70 wt % $HCCl_2COOH$ solution and (B) in 82 wt % $HCCl_2COOH$ solution. The horizontal dotted lines indicate the values of τ_{exp} described in the text.

Discussion

The helix-coil transition in a mixed solvent has been treated by several authors.[40-44] From all of these theories, it appears that the Zimm-Bragg theory can be applied directly to the experimental data in the mixed solvent, as for a polymer in a pure solvent, but that the resulting thermodynamic parameters include the binding (and other) effects of the solvent. Most importantly, for comparison with the calorimetric data in the accompanying paper,[11] the observed values of $\Delta H°$ (for the conversion of a coil to a helical residue) obtained from the ORD and calorimetric measurements should be identical since they both contain the same contributions from the solvent.

The experimental data in the form of θ_h *vs.* T were taken at intervals of 5° from the smoothed experimental

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(37) The lower-$\overline{DP}$ samples gave more positive values of b_0, *viz.*, $b_0 = -624$ and -590 for fractions III-3 ($\overline{DP}$ = 700) and IV-3 ($\overline{DP}$ = 130), respectively. b_0 was found to be independent of temperature (between 25 and 60°) and concentration. Other values reported in the literature range from $b_0 = -630$ to -660,[38,39] without specification of chain length.

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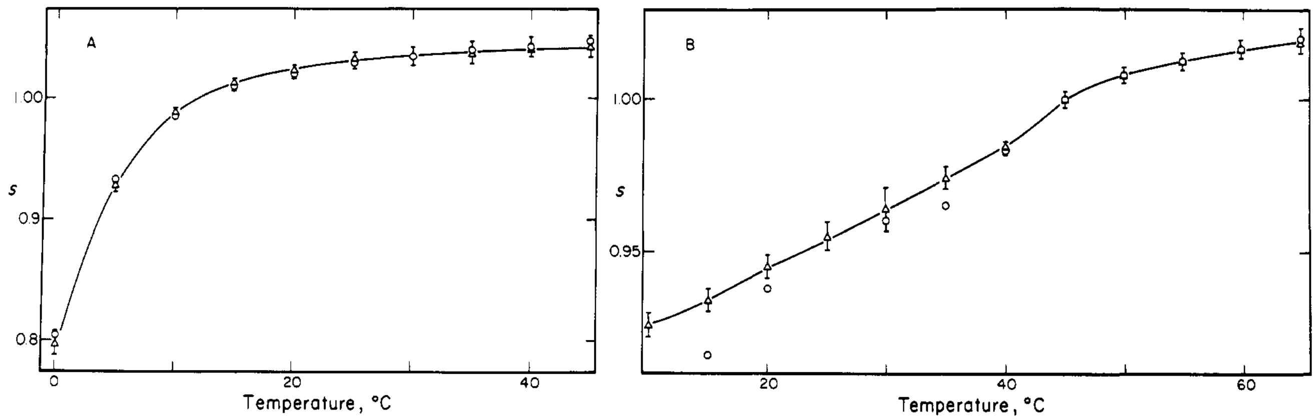

Figure 4. Plot of *s vs. T.* (A) in 70 wt % $HCCl_2COOH$ solution and (B) in 82 wt % $HCCl_2COOH$ solution. The triangles and circles represent *s* values obtained using temperature-independent and -dependent values of σ, respectively. The error limits (obtained as described in the text) are shown by vertical lines.

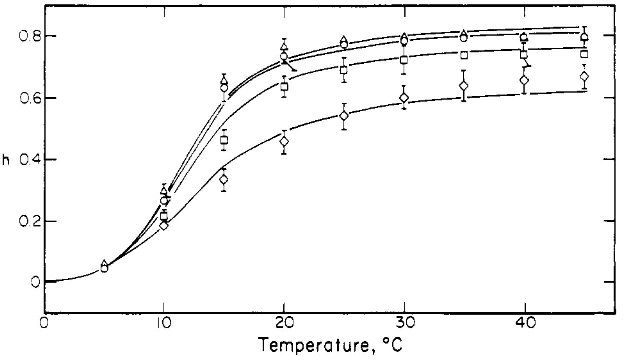

Figure 5. Plot of θ_h *vs. T* in 70 wt % $HCCl_2COOH$ solution. The points are experimental and the curves are theoretical. The symbols representing the fractions are the same as in Figure 1. The error limits were obtained as described in the text.

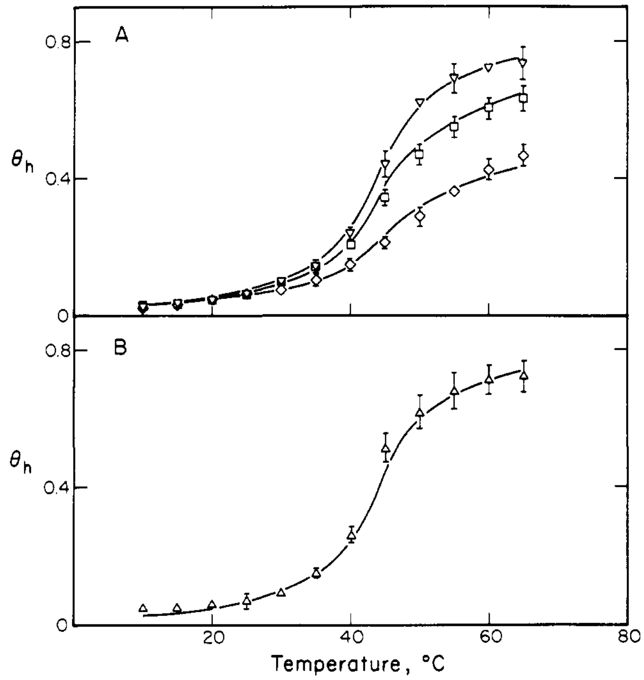

Figure 6. Plot of θ_h *vs. T* in 82 wt % $HCCl_2COOH$ solution. The points are experimental and the curves are theoretical. The symbols representing the fractions in (A) and (B) are the same as in Figure 2A,B, respectively. The error limits were obtained as described in the text.

curves of Figures 1 and 2, and were used to compute the helix–coil transition parameters for PBLG in the two compositions of the solvent mixture. The Zimm–Bragg theory[10] was employed for this purpose. The theoretical analysis of the data was performed in the same manner as described in detail in our earlier papers.[3-9,45] Thus, two procedures of calculation were employed, one in which the parameter σ was treated as temperature independent and another where it was taken as temperature dependent. In the former method, for each of several trial values of σ, the best value of the parameter *s* at each temperature was computed as the one that minimized the quantity τ which measured the goodness of the fit of the theoretically calculated values of θ_h with the experimental ones for all the fractions at this temperature. The quantity τ has been defined earlier[4] as

$$\tau = \sum_T \sum_i [(\theta_{h,expt})_{i,T} - (\theta_{h,calcd})_{i,T}]^2 \quad (1)$$

where the two quantities inside the brackets are, respectively, the experimental and calculated (theoretical) values of θ_h for the *i*th fraction (of a given $\overline{DP}$) at the temperature *T*. By taking the summation over all temperatures, one obtains the best value of σ as the one which yields the minimum value of τ. Plots of τ *vs.* σ in the 70 and 82 wt % $HCCl_2COOH$ solutions are shown in Figure 3. It is seen that the best values of σ are 3.5×10^{-4} in 70 wt % $HCCl_2COOH$ and 2.0×10^{-4} in 82 wt % of $HCCl_2COOH$ solutions. The error in these values of σ was determined by computing a quantity τ_{exp}[6,8] which is given by

$$\tau_{exp} = \sum_i \sum_T [(\Delta\theta_{h,calcd})_{i,T} + (\Delta\theta_{h,expt})_{i,T}]^2 (\theta_{h,expt})_{i,T}{}^2 \quad (2)$$

where $(\Delta\theta_{h,calcd})_{i,T}$ is a fractional quantity which contains the error in θ_h arising from the error in the determination of chain length and $(\Delta\theta_{h,expt})_{i,T}$ is a similar quantity containing errors in (a) the estimate of $b_0{}^H$ and $b_0{}^C$, (b) the slope of the Moffitt–Yang plot, and (c) the PBLG concentration. The values of σ which fell within the respective values of τ_{exp} in Figure 3 were taken to represent the deviations of the values of σ in the two solutions. Thus, in the 70 wt % $HCCl_2COOH$ solution, the allowed values of σ were found to lie between about 2×10^{-4} and 9×10^{-4}, while in 82 wt % $HCCl_2COOH$ solution, σ varied from about 1×10^{-4} and 4×10^{-4}.

Plots of *s* as a function of temperature are shown in Figure 4 for the 70 and 82 wt % $HCCl_2COOH$ solutions, using the temperature-independent values of σ given above. The error limits on the values of *s* were computed as described previously[6] using the two sets of data characterized by $\theta_{h,max}$ and $\theta_{h,min}$ (which represent the maximum and

[45] The computer programs used in these calculations are available; see footnotes 26 and 27 of ref 3.

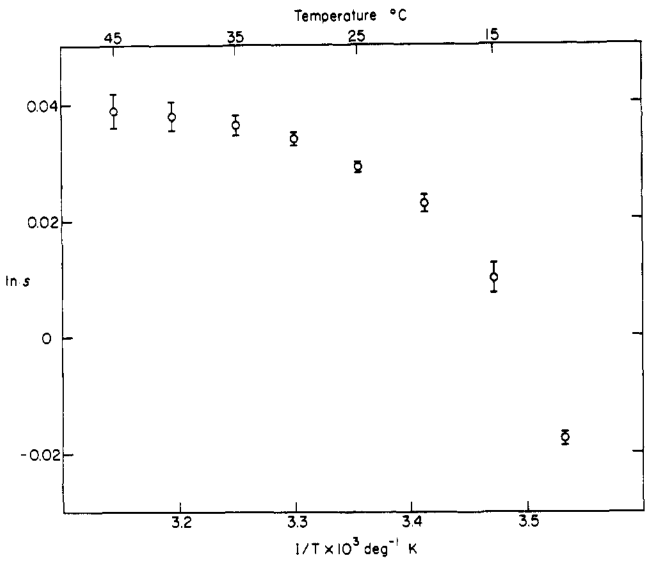

Figure 7. Plot of ln s *vs.* $1/T$ for PBLG in 70 wt % $HCCl_2COOH$ solution. The vertical lines represent the error limits (see text).

minimum values, respectively, of θ_h at a given temperature and chain length computed by adding to or subtracting from it the total error in θ_h). The error limits in s obtained in this manner are shown in Figure 4.

In the second method of analysis of the data, σ was allowed to vary with temperature so that at each temperature best values of both σ and s were obtained. However, the variations in σ over the experimental temperature range were within the limits of error of the temperature-independent σ derived earlier in both 70 and 82 wt % $HCCl_2COOH$. The values of s at various temperatures computed with the temperature dependent σ values are also shown in Figure 4. Except for some points at low temperature in 82 wt % $HCCl_2COOH$ solution, these values of s lie within the limits of error of those computed with a temperature-independent σ. Therefore, within the limits of the experimental errors, it is not possible to detect the temperature dependence, if any, of the parameter σ.

The theoretical values of θ_h as a function of temperature have been calculated using the temperature-independent best values of σ and the data of s *vs.* T in Figure 4. These are shown in Figures 5 and 6, along with the experimental data. The agreement between theory and experiment is found to be satisfactory in both 70 and 82 wt % $HCCl_2COOH$ solutions, taking into account the experimental errors shown in Figures 1 and 2 (which, in fact, do not include errors in chain length and are therefore smaller than the true errors).

Use was made of the temperature dependence of s, in both solvent compositions, to evaluate the changes in free energy, $\Delta G°$, enthalpy, $\Delta H°$, and entropy, $\Delta S°$, for the conversion of a coil residue (at the end of a long helical sequence) to a helical one by means of

$$\frac{\mathrm{d}\ \ln s}{\mathrm{d}(1/T)} = \frac{-\Delta H°}{R} \qquad (3)$$

Figures 7 and 8 show the plots of ln s *vs.* $1/T$ for the 70 and 82 wt % $HCCl_2COOH$ solutions, respectively. The error symbols shown in these figures are derived from the errors in the determination of the values of s shown in Figure 4. The data in 70 wt % $HCCl_2COOH$ solution below 10° are not shown in Figure 7 because θ_h is close to zero, and the resulting errors in θ_h are very large.

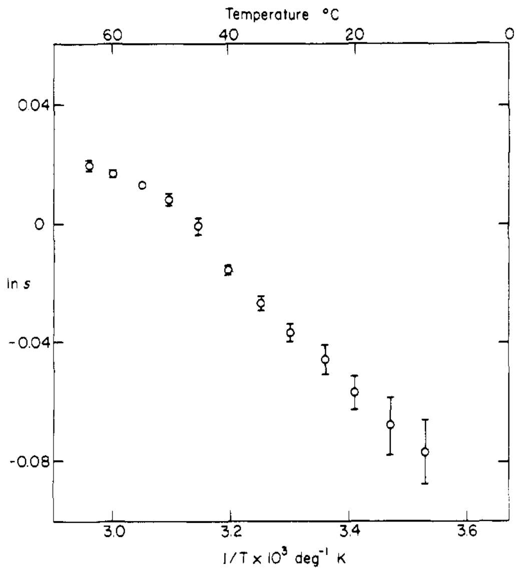

Figure 8. Plot of ln s *vs.* $1/T$ for PBLG in 82 wt % $HCCl_2COOH$ solution. The vertical lines represent the error limits (see text).

Table II
Thermodynamic Parameters for the Helix-Coil Transition of PBLG in $HCCl_2COOH$-Cl_2Et Mixtures

	70 wt % $HCCl_2COOH$	82 wt % $HCCl_2COOH$
$\Delta G_{25°}$ (cal/mol)[a]	-17 ± 4	27 ± 3
$\Delta H°$ (cal/mol)	770 ± 50[b]	360 ± 15[c]
$\Delta S°$	2.7 ± 0.2[b]	1.12 ± 0.04[c]
T_m (°C[d])	14 ± 2	45 ± 2
σ[e]	0.00035	0.0002

[a] Taken from data in Figures 7 and 8. [b] Calculated at T_m as described in the text. [c] Calculated average value between 0 and 65° (see text). [d] Calculated from data in Figure 4 at $s = 1$. [e] Limits of error in σ are shown in Figure 3.

The data in Figure 8 for the 82 wt % $HCCl_2COOH$ solution were fit well within the limits of error to a weighted least-squares straight line,[46] so that $\Delta H°$, which is obtained from the slope of this line (eq 3), appears to be independent of temperature in this case. However, this was not true for the 70 wt % $HCCl_2COOH$ solution, since the plot shown in Figure 7 shows a marked curvature indicative of a strong temperature dependence of ΔH in this solvent composition. The values of $\Delta H°$ can be obtained from the slope of the curve in Figure 7 at each temperature. At temperatures greater than 25°, the variation in θ_h (or ln s) with temperature is very small, making it difficult to estimate ΔH above 25°. In the range of 10-25°, $\Delta H°$ was found to vary from about 900 cal/mol (at 10°) to about 200 cal/mol (at 25°). A value of 770 ± 50 cal/mol was obtained from the slope of the curve in Figure 7 at T_m (= 14°) (where ln s = 0), and is given in Table II along with the value of $\Delta S°$, for PBLG in 70 wt % $HCCl_2COOH$ solution; the temperature-independent values of $\Delta H°$ and $\Delta S°$ in 82 wt % $HCCl_2COOH$ solution are also shown in Table II.[47]

To facilitate the comparison of our results shown in Table II with similar data obtained by others, the available information on the thermodynamic parameters for the PBLG-$HCCl_2COOH$-Cl_2Et system as obtained by polarimetric measurements are collected together in Table III. In general, our values of σ lie in the range found by

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(46) P. R. Bevington, "Data Reduction and Error Analysis for the Physical Scientist," McGraw-Hill, New York, N. Y., 1969, p 92.

(47) It must be emphasized that $\Delta H°$ and $\Delta S°$ contain contributions from all solvent effects (including binding) as well as from transition in the absence of binding.

Table III
Reported Values of the Thermodynamic Parameters for PBLG in HCCl$_2$COOH-Cl$_2$Et Mixtures from Polarimetric Measurements

Solvent Composition (wt % HCCl$_2$-COOH)	PBLG Concn % (w/v)	Chain Length ($\overline{\mathrm{DP}}$)	$\sigma \times 10^4$	$\Delta H°$ (cal/mol)	$\Delta S°$ (eu)	T_m (°C)	Ref
69.8	0.7-1.0	1900	0.9 ± 0.25	800 ± 50	*a*	5.1	22
		580					
		250					
		145					
70.0	*a*	1500	2.0 ± 0.6	890 ± 130	*a*	11.8	14
		46					
		26					
70.0	1.0	1120	1.96	890	*a*	11.5	21
70.0	0.46-2.0	1000	3.5	770 ± 50	2.7 ± 0.2	14	This work
		700					
		320					
		130					
74.4	0.5-0.8	1900	0.9 ± 0.25	780 ± 50	*a*	15.2	22
		580					
		250					
		145					
81.0	1.0	1120	1.96	840	*a*	31.6	21
81.5	0.5	1900	0.9 ± 0.25	580 ± 60	*a*	36.3	22
		580					
		250					
		145					
82	0.36-4.0	1550	2.0	360 ± 15	1.12 ± 0.04	45	This work
		1000					
		320					
		130					

[a] Data not given.

others, but our values of $\Delta H°$ are somewhat lower than those reported for the same solvent compositions. As possible reasons for this discrepancy, we note that, first of all, several well-fractionated samples of appropriately spaced value of $\overline{\mathrm{DP}}$ are required for a good determination of the thermodynamic parameters. Only one (unfractionated) sample was used in the work of ref 21, and the DP 26 and 46 samples of ref 14 were too short for obtaining a high enough helix content; also heterogeneity in chain length could be very serious at such low values of $\overline{\mathrm{DP}}$. Secondly, instead of using ORD (and evaluating b_0 to obtain θ_h), the values of θ_h were obtained from the observed optical rotation at a single wavelength in ref 14, 21, and 22. As will be shown in the accompanying paper,[11] the values of $\Delta H°$ obtained by calorimetric measurements compare fairly well with those reported in this paper from ORD measurements.

The values of $\Delta H°$ obtained here from ORD measurements, and from calorimetric measurements in the accompanying paper,[11] are independent of the concentration of PBLG over the range studied (about 0.4–4.0%, w/v). These observations disagree with the polarimetric results of Puett and Ciferri[23] (who used CHCl$_3$ instead of Cl$_2$Et) and the calorimetric results of Ackermann and Neumann[17] (who used HCCl$_2$COOH-Cl$_2$Et mixtures).[48,49]

Finally, we consider the possible usefulness of PBLG in the two solvent compositions studied here as the "host" polypeptide in a copolymer study, as indicated earlier. Table IV lists the values of the parameters σ and s at 25° for PBLG in 70 and 82 wt % HCCl$_2$COOH solutions as well as those of poly(hydroxybutylglutamine) (PHBG) and poly(hydroxypropylglutamine) (PHPG) in water, the latter two[4] having been employed as "hosts" in our earlier investigations in water[5-9] to obtain the thermodynamic parameters for "guest" residues such as, glycine, alanine, etc., which were incorporated in random copolymers with the respective host residues. The data in Table IV reveal that benzyl L-glutamate can act either as a helix-making ($s > 1$) or helix-breaking ($s < 1$) residue at 25° depending on the solvent composition used. In fact, the values of s and σ at 25° for PBLG in 70 wt % HGCl$_2$COOH are very close to those of PHBG in water at this temperature, so that, like the latter, PBLG in 70 wt % HCCl$_2$COOH solution can be employed as host for potential helix-breaking guest residues, as has been demonstrated in a study of the copolymer of PBLG and glycine in this solvent mixture.[50] Since the helix-coil transitions in HCCl$_2$COOH-Cl$_2$Et mixtures are inverted (unlike the case in aqueous solution[4-9]), the incorporation of a helix-breaking guest residue like glycine in the copolymer containing PBLG in 70 wt % HCCl$_2$COOH solution, causes the T_m to be shifted progressively to higher temperatures as the content of the

Table IV
Values of the Zimm-Bragg Parameters at 25° for "Host" Polypeptides

Polypeptide	Solvent	$\sigma \times 10^4$	s
PBLG	70 wt % HCCl$_2$COOH in Cl$_2$Et	3.5	1.03
PBLG	82 wt % HCCl$_2$COOH in Cl$_2$Et	2.0	0.96
PHBG	Water	6.7	1.02
PHPG	Water	2.2	0.98

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(48) After completion of this manuscript, our attention was drawn to the recent paper of Teramoto and Norisuye[49] who also find no concentration dependence of $\Delta H°$.

(49) A. Teramoto and T. Norisuye, *Biopolymers*, 11, 1693 (1972).

(50) V. S. Ananthanarayanan, E. Leroy, and H. A. Scheraga, to be published.

guest residue in the copolymer is increased.[50] In a similar manner, PBLG in 82 wt % $HCCl_2COOH$ solution, which is the counterpart of PHPG in water, can be used as the host residue in copolymers containing potential helix makers as guest residues.

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Helix–Coil Transition in Mixed Solvents. II. Calorimetric Study of Poly(γ-benzyl L-glutamate) in Dichloroacetic Acid–Dichloroethane Mixtures[1]

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ABSTRACT: The enthalpy change, ΔH°, for the helix-coil transition of poly(γ-benzyl L-glutamate) in mixtures containing 70:30 and 82:18 weight ratios of dichloroacetic acid and dichloroethane, respectively, has been determined with the aid of a differential thermal analysis apparatus. The description of the apparatus and the details of analysis of the data are given. The values of ΔH° obtained in the two solvents compare fairly well with those computed from spectropolarimetric measurements in the preceding paper. Also, within the limits of the experimental error, it appears that ΔH° is independent of the concentration of the polypeptide over the range of concentrations studied.

In the preceding paper,[3] we indicated how we plan to use poly(γ-benzyl L-glutamate) (PBLG) as a host polymer in mixtures of dichloroacetic acid ($HCCl_2COOH$) and dichloroethane (Cl_2Et) for determining the Zimm-Bragg parameters[4] σ and s of guest amino acid residues in nonaqueous solvents. To apply the host-guest technique it is first necessary to determine the thermodynamic parameters for the thermally-induced helix–coil transition in the host homopolymer. This was done[3] for PBLG in $HCCl_2COOH$-Cl_2Et mixtures, by use of optical rotatory dispersion (ORD) measurements, and we report here a calorimetric determination of the enthalpy change (ΔH°) for the same (inverted) transition, and compare it to the value obtained[3] from the temperature dependence of s.

Several calorimetric evaluations of ΔH° for the PBLG–$HCCl_2COOH$-Cl_2Et system have already been reported.[5-13] The motivation for this additional investigation is (1) to obtain independent data on the same systems used in our ORD study[3] to establish the thermodynamic parameters for the host homopolymer prior to further studies on the host-guest random copolymers, and (2) to demonstrate the applicability of a recently developed differential thermal analysis (DTA) apparatus for obtaining precise values of ΔH° for thermally induced helix-coil transitions. The DTA apparatus used here is of the conduction type,[14,15] and thus is simple in design and operation, and is low in cost compared to adiabatic-type calorimeters. The heat detectors used in our calorimeter are semiconductor thermoelectric generators and are superior to the thermopiles consisting of many wire-type thermocouples, used in earlier conduction-type calorimeters.[15] By making use of twin cells we have gained the advantages of a differential heat measurement. Another advantage of our calorimeter is that only about 40 mg of polypeptide (in $\sim$1.5 ml), approximately $^1/_{100}$th of the size sample usually used,[7] is required for good precision. These advantages have been gained without sacrificing precision and accuracy; as will be shown by the results reported here, the precision and accuracy in the measurement of heat change is comparable to those obtainable with an adiabatic-type calorimeter.[16]

The DTA apparatus is described, and then results are presented for the helix-coil transition in PBLG in 70 and 82 wt % $HCCl_2COOH$, respectively, in Cl_2Et.

Experimental Section

Materials. The PBLG samples were fractions I-2 and II-2 of the previous paper;[3] these have average degrees of polymerization ($\overline{DP}$) of 1550 and 1000, respectively. $HCCl_2COOH$ and Cl_2Et were the same solvents as in the earlier study.[3] *n*-Hexadecane was of Spectrophotometric grade from Aldrich Chemical Co.

The solutions of PBLG were prepared as described earlier,[3] and the concentration of polymer was determined by dry weight.

Description of Calorimeter. A schematic drawing of the calorimeter is shown in Figure 1A,B. The sample and reference cells (C_1 and C_2, respectively) were type 37 microcylindrical spectrophotometric glass cells, with long filling tubes, from Precision Cells Inc., New York. The separation of the internal faces of the cells was 5 mm and the internal diameter was 22 mm; the filling tubes were long (7 cm) and narrow (about 2-mm i.d.) to minimize evaporation of cell contents. The masses of the cells were matched to within 0.1 mg by grinding the faces of the cell. The

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